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Citrus Nematode Biotypes and Resistant Citrus Rootstocks in Florida[1]

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INTRODUCTION: *Tylenchulus semipenetrans* Cobb, 1913 (Fig. 1A), has a worldwide geographical distribution (O'Bannon and Ford 1977). It is the most common plant parasitic nematode of citrus in citrus groves. Although most *Citrus* species are preferred hosts of this parasite, citrus relatives (Rutaceae family), such as *Poncirus trifoliata* (L.) Raf. and its hybrids, can be parasistized as well (Fig. 1B). Citrus nematodes also infect non-rutaceous plants, such as grape (*Vitis vinifera* L.) (Raski *et al.* 1956), olive (*Olea europaea* L.) (Baines and Thorne 1952), and persimmon (*Diospyrus* spp.) (Raski *et al.* 1956; Cohn and Minz 1961). There is no evidence that the citrus nematode infects herbaceous plants. Due to host differentiation, three biotypes of this nematode are recognized: citrus, mediterranean and poncirus (Inserra *et al.* 1980; Gottlieb *et al.* 1986; Verdejo-Lucas 1992) (Table 1). These *T. semipenetrans* biotypes share citrus species as a common host, but differ in their ability to infect and reproduce on *P. trifoliata* and olive. *Poncirus trifoliata* is only parasitized by the poncirus biotype. Olive is infected only by the citrus biotype. The mediterranean biotype reproduces very poorly on *P. trifoliata* and does not infect olive (Table 1).

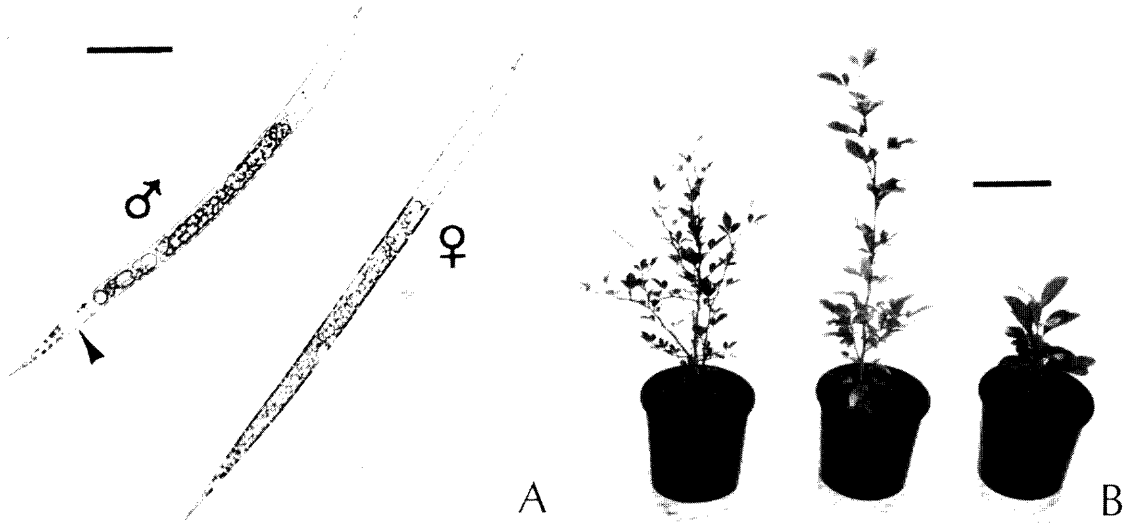

Fig. 1. Citrus nematodes and seedlings of citrus rootstocks used by the citrus industry. Scale bars = 48 µm in A and 10 cm in B. A) Second-stage juvenile (J2) male and female. Note in the male posterior body a clear and square-like area (arrow), which differentiates J2 males from females. B) From left to right, trifoliate orange, Swingle citrumelo and rough lemon seedlings. (Photography: R. N. Inserra and J. Lotz).

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Table 1. Host preferences of *Tylenchulus semipenetrans* biotypes.

	Nematode Biotypes[a]		
	Citrus	Mediterranean	Poncirus
Citrus (*Citrus* sp.)	+	+	+
Grape (*Vitis vinifera*)[b]	+	+	+
Olive (*Olea europaea*)	+	†	†
Persimmon (*Diospyrus lotus*)[b]	+	+	unknown
Trifoliate orange (*Poncirus trifoliata*)	-	-	+

[a] Symbols + and - indicate good or poor nematode reproduction, respectively. Symbol † indicates no infection.
[b] Other *Vitis* and *Diospyrus* species may differ in their reaction to *T. semipenetrans* biotypes.

The distribution of citrus nematode biotypes is not well defined. From the results of host preference studies reported in the literature, it appears that the citrus biotype occurs in California and Italy (Baines *et al.* 1974; Inserra *et al.* 1980). The mediterranean biotype has been reported from countries in the Mediterranean basin and South Africa (Inserra *et al.* 1980; Gottlieb *et al.* 1986; Verdejo-Lucas 1992). The poncirus biotype is present in California, Israel and Japan (Baines *et al.* 1974; Gottlieb *et al.* 1986). The citrus nematode populations from Florida, Arizona and Texas may be either citrus or mediterranean biotypes, because they infect and reproduce poorly on *P. trifoliata.* Their infection potential on olive trees is unknown (O'Bannon *et al.* 1977). The citrus nematode populations that occur in Argentina, Australia, Brazil, India, and Venezuela may be either the citrus or mediterranean biotypes because they reproduce poorly on *P. trifoliata* and have not been tested on olive (Crozzoli and Funes 1992; Campos and Ferraz 1980; O'Bannon and Ford, 1977).

EFFECT OF CITRUS ROOTSTOCKS ON THE SELECTION AND DISTRIBUTION OF *T. SEMIPENETRANS* BIOTYPES: Citrus and mediterranean biotypes are probably more widely distributed than that of the poncirus biotype, because rootstocks of the genus *Citrus*, such as limes [*C. aurantifolia* (Christm.) Swingle], rough lemon [*C. limon* (L.) Burm. f.] and sour orange (*C. aurantium* L.) have been more commonly used worldwide than *P. trifoliata,* except for Japan. The adoption of *P. trifoliata* as a major rootstock in Japan and the successive introduction of this rootstock and its resistant hybrids in other parts of the world [California, Mediterranean basin (Israel)] has induced the selection and possibly the dissemination of the poncirus biotype. *T. semipenetrans* populations are not known to attack *P. trifoliata* in the majority of the citrus-growing areas in countries of the Mediterranean basin and South America, and in South Africa, Arizona, Florida and Texas where *P. trifoliata* and its resistant hybrids (X *Citroncirus*) have only recently been used as rootstocks (O'Bannon *et al.* 1977). In those areas, which are free of the poncirus biotype of the nematode, *P. trifoliata* lines have also been used as a source of resistance to the citrus nematode in the selection of hybrids between *Citrus* species and *P. trifoliata.* These hybrids often lack the undesirable agronomic characteristics of *P. trifoliata* such as poor vigor and sensitivity to high pH soils, but sometimes retain resistance to citrus tristeza virus, foot rot (*Phytophthora parasitica* (Dastur) Waterh.) and the citrus nematode. Some of these hybrids have been recommended to replace commonly used citrus rootstocks, such as rough lemon in Florida (Hutchinson *et al.* 1972). However, only a few poncirus hybrids have inherited resistance to the citrus nematode. The hybrid Carrizo citrange, which has been used in Florida for its tolerance to the burrowing nematode, is not resistant to the citrus nematode.

Among the citrumelo hybrids (*C. paradisi* Macf. X *P. trifoliata*) only Swingle citrumelo was reported to be highly resistant to the citrus nematode (O'Bannon and Ford 1977). Swingle citrumelo was obtained by W. S. Swingle at Eustis, Florida, in 1907. The promising agronomic characteristics of this rootstock were reported by Hutchinson (1974). Its resistance to *T. semipenetrans* was demonstrated under field and greenhouse conditions in Florida (O'Bannon and Ford 1977; Kaplan and O'Bannon 1981). This rootstock is also resistant to other citrus nematode populations outside Florida, such as those from Italy (Lo Giudice and Inserra 1980), Venezuela (Crozzoli and Funes 1992), and probably Brazil (Campos and Ferraz 1980). In Florida, Swingle citrumelo has been widely used in new citrus-growing areas and in replantings of old citrus groves.

CITRUS NEMATODE POPULATIONS ATTACKING SWINGLE CITRUMELO IN FLORIDA: In 1992, Swingle citrumelo plants, which were infected by large numbers of the citrus nematode, were detected in Pasco County in a nursery which failed to meet the Department of Agriculture and Consumer Services certification requirements for commercial citrus nurseries. Nematode root densities in some of these seedlings were > 600 swollen females/g fresh root and did not differ from those of other susceptible rootstocks present in the site. The cause of the nematode infection on the resistant Swingle citrumelo in this nursery of Pasco County has been the subject of field and greenhouse studies for about two years (Duncan *et al.*, in preparation). The aim of these studies was to verify if a new resistance-breaking biotype of *T. semipenetrans* was present in the nursery or if variability of Swingle citrumelo germplasm was the cause of the nematode infection. Site inspections, where the nematode-infected Swingle citrumelo seedlings were originally grown, excluded the possibility of nematode infection via the seedbeds or the introduction of propagating material infected by the poncirus biotype of the nematode from unknown sources, outside Florida.

In two concomitant field studies, germplasm certified as Swingle citrumelo, *P. trifoliata*, and rough lemon seedlings were planted and grown in the nursery site infested with the potential resistance-breaking biotype of the nematode in Pasco County (site 1) and also in a citrus orchard infested with the citrus or mediterranean biotypes (infecting and reproducing poorly on *P. trifoliata* and Swingle citrumelo) in Polk County (site 2). The results of the field experiments indicated that the two populations from the two orchards differed in their ability to infect Swingle citrumelo and *P. trifoliata* seedlings. At site 1, infested with the new potential resistance-breaking biotype of the nematode, nematode root densities on Swingle citrumelo did not differ from those observed on a susceptible rootstock rough lemon. At site 2, infested with the citrus or mediterranean biotype of the nematode, *T. semipenetrans* numbers were significantly smaller on Swingle citrumelo and *P. trifoliata* roots, compared to those detected on the susceptible rough lemon (Duncan *et al.*, in preparation).

Results from subsequent greenhouse tests, using the same citrus rootstocks and nematode populations from sites 1 and 2, confirmed those of the field tests. These preliminary trials provide strong evidence that the population in Pasco County can easily parasitize the previously believed citrus nematode-resistant rootstocks Swingle citrumelo and *P. trifoliata*. The population from Polk County did not readily parasitize these two rootstocks. However, in the field experiment some parasitization by the nematode population from Polk County occurred on Swingle citrumelo and *P. trifoliata*. This slight infection was favored by the continuous exposure of the two resistant rootstocks to the high number of citrus nematodes released from the infected root systems of the old citrus trees. Further trials are necessary to verify if the population from Pasco County will behave as the poncirus biotype of the citrus nematode.

SURVEY AND DETECTION: *So far there is no evidence that resistance-breaking populations of the citrus nematode occur commonly in Florida citrus orchards.* The presence of a resistance-breaking biotype of *T. semipenetrans* in Pasco County appears to be confined to the nursery site under investigation. The site approval and citrus nursery certification programs implemented by the Florida Department of Agriculture and Consumer Services prevent any movement and consequent dissemination of citrus seedlings infected by the citrus nematode from citrus nurseries into new planting sites. Furthermore, the nematode has not been found infecting native wild plants of Florida. Thus, it is unlikely that new biotypes of *T. semipenetrans* will be found in new citrus-growing areas. Old citrus orchards with records of citrus nematode infestations should be sampled if Swingle citrumelo or *P. trifoliata* rootstocks have been introduced to replace nematode-susceptible rootstocks, such as rough lemon or sour orange. Samples containing soil and roots of Swingle citrumelo or *P. trifoliata* should be collected from any suspect sites and submitted for nematological analysis to the Nematology Section of the Florida Department of Agriculture and Consumer Services, Division of Plant Industry.

CONCLUSIONS: Swingle citrumelo is an excellent rootstock that is highly resistant to the citrus nematode in Florida. The use of this rootstock should be encouraged in all new citrus-growing areas of the state. However, sites heavily infested by the citrus nematode and replanted to Swingle citrumelo or *P. trifoliata* should be monitored to observe whether selection pressure results in the selection of resistance-breaking populations of the parasite. Interplanting Swingle citrumelo with infected susceptible citrus trees can expose the Swingle citrumelo root system to continuous invasion of high densities of the nematodes which may overcome the resistance of this rootstock. This phenomenon was observed in the field experiment in the citrus orchard in Polk County (Duncan *et al.*, in preparation). It would be beneficial to the Division of Plant Industry and the citrus industry to know the sites where Swingle citrumelo and *P. trifoliata* are interplanted among susceptible rootstocks in citrus nematode-infested orchards.